American Heart Association®
Fighting Heart Disease and Stroke

National Center
7272 Greenville Avenue
Dallas, Texas 75231-4596
Tel 214.373.6300
americanheart.org

22 May 2002

The European Patent Office
Erhardtstrasse 27
D-80298 Munich
Germany

Dear Sirs

I have been asked to comment on the procedure, whereby abstracts for the AHA's annual conference are published, with particular reference to 1996.

The abstracts are collected and printed in advance of the conference. However, they are not sent out to delegates. The collection is given to participants only when they arrive at the meeting, in exchange for a voucher that they receive when paying their registration charges. Further, there is a news embargo on all data in the abstracts until their oral presentation. This is consistent with the AHA's policy of ensuring that the presentations have the desired impact, and avoiding advance speculation on the content of presentations.

This policy was no different in 1996. The abstracts for that year's conference were printed in October, but were not available to delegates, or any other member of the public, until the conference itself, in November.

Sincerely,

Richard L. Luna
Scientific Sessions Abstract Coordinator
American Heart Association

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

Our Reference BB-IND 11010

MAY 3 0 2003

Ark Therapeutics Limited
Attention: James M. Parker, Ph.D.
Strategic Bioscience Corporation
93 Birch Hill Road
Stow, MA 01775-1308

Dear Dr. Parker:

We have reviewed your **Investigational New Drug Application (IND)** for "Adenovirus Vector (Ad-VEGF-D, Cobra-Bio Manufacturing) Expressing Human Vascular Endothelial Growth Factor D Gene; Delivered via Collagen Collar Device." As discussed during the telephone conversation of May 2, 2003 between you and Dr. Maritza McIntyre of this office, your proposed study under this IND has been placed on clinical hold.

Your IND is on clinical hold because insufficient information has been submitted to allow FDA to assess the risks to the subjects in the proposed clinical investigation [21 CFR 312.42(b)(1)(iv)]. Specifically, we have the following comments and requests for information:

PRODUCT INFORMATION

Your description of endotoxin testing indicates that the sample used for testing is removed prior to the final filling. Lot release testing for endotoxin and sterility should be performed on the final vialed product. Please perform endotoxin tests on samples of Trinam™ lots CTL2002#132U and CTL2002#991E taken after the filling operation is completed. In addition, endotoxin testing must be performed on vialed samples from Trinam™ lots CTL2002#990E and CTL2002#989E, which were derived from lot CTL2002#991E by dilution. Please be aware that you may not use any of these lots in your clinical protocol until these tests have been performed and meet lot release testing specifications.

2 Regarding the RCA testing:

a. Please provide a better description of the assay methodology to ensure that the amount of replication competent adenovirus in $3x10^{10}$ virus particles is measured.

b Specifically, please describe how the virus particle number is being tested, since this test is performed on the crude virus lysate (CVL) and the sample is diluted to avoid cytotoxicity.

c. We acknowledge your commitment not to release Trinam™ lot CTL2002#132U until RCA testing has been performed on a sample of the CVL from which this lot was derived and the sample found to meet specifications. Please acknowledge that retention samples from this CVL are available to perform this assay.

3. Regarding the *in vivo* and *in vitro* tests for adventitious viral agents, please provide a better description of the assay methodology which includes the degree of dilution of the test article. In addition, we note that the *in vitro* test for adventitious viral agents was not performed on the CVL from which Trinam™ lot CTL2002#132U was derived (see batch analysis results, Table 7-1A.20, section 7-1, p.85). Please be aware that you may not use this lot in your clinical trial until this test has been performed and the sample found to be free of adventitious viral agents. Please acknowledge that retention samples from this CVL are available to perform this assay.

4. Regarding the manufacture, testing, and release of the final product, please note that as sponsor of the IND, you are responsible for ensuring that all steps are performed according to standard operating procedures (SOPs), that the assays are qualified for the product being tested, and that appropriate quality assurance review of manufacturing and testing is conducted before the release of the product, regardless of who is actually responsible for performing those functions. Please provide a description of the quality assurance program in place at Ark Therapeutics, Ltd to ensure that the contractor is following appropriate procedures.

5. We note that signed certificates of analysis (CoAs) have been generated by the quality assurance (QA) unit of Cobra Bio-Manufacturing, which has responsibility for final product release, for Trinam™ lots CTL2002#132U although RCA and *in vitro* virus testing has not been performed and for lots CTL2002#989E, CTL202#990E, and CTL#991E, although the results for sterility testing are pending. The CoAs are not labeled in any way to suggest that the product listed is pending release for clinical use. We recommend that a system is put in place to prevent issuance of signed CoAs for drug products which have not undergone full lot release testing. Please comment.

CLINICAL INFORMATION

6. Your product requires surgical application of a pervascular biodegradable collagen device into which test material containing the adenoviral vector with transgene product is injected. This is a highly specialized procedure. The control procedure is standard of care placement of polytetrafluoroethylene (PTFE) graft. Please submit the following information:

a. Because the procedure you propose is highly specialized, we request that you include the names and credentials, including the curriculum vitae, for all surgeons who are planned to perform both the control and test operative procedures. For example, you have listed Aamir Zakaria, M.D. as a subinvestigator in this study, but his curriculum vitae (CV) is not included in your submission. Please submit his CV and indicate whether he will perform the operative procedures.

b. Please submit the information concerning the method of training that you will provide for the surgeons who are planned to perform the procedure to ensure that the procedures will be performed in a standardized fashion.

7 Please exclude subjects who may be at increased risk for adverse events if they receive a product containing an adenoviral vector or the vascular endothelial growth factor-D (VEGF-D) transgene. We have the following comments regarding the eligibility criteria:

a. The National Institutes of Health Recombinant DNA Advisory Committee (RAC) reviewed your protocol on September 6, 2001. The RAC recommended exclusion of subjects with Grade 1 or 2 liver toxicity because of possible hepatotoxicity of the adenoviral vector. Please revise your protocol accordingly.

b. Subjects who are immunosuppressed may have increased susceptibility to adverse events of adenovirus infection. Please exclude subjects who are receiving steroids or other immunosuppression medications for any reason Please incorporate this revision into your protocol.

c. VEFG-D has been associated with neoplastic growth. Please exclude subjects with any history of cancer, except non-melanoma skin cancers. Please revise your eligibility criteria to reflect this risk.

d. Subjects should be screened for colon cancer following the American Cancer Society (ACS) Guidelines prior to enrolling in the trial. For example, ACS Guidelines recommend that subjects over age 50 with no other risk factors for colon cancer have three stool samples for examination for fecal occult blood within a year of enrollment and a colonoscopy within ten years of enrollment or sigmoidoscopy within five years of enrollment. Please revise your screening procedures to include these tests and revise the protocol to exclude subjects who have positive tests.

e Your first inclusion criterion is that subjects be undergoing placement of a PTFE arteriovenous hemodialysis arm graft. You do not state the etiology of the renal failure that is the basis for the subject requiring dialysis. Conceivably, different etiologies of renal failure may convey different risks involved in undergoing this procedure and may predispose to different outcomes concerning the graft function, either due to the underlying condition or the treatment for that condition (e.g., steroids or other immunosuppressive agents). In your eligibility criteria, please include a list of conditions or exemplary conditions that would be considered for inclusion or exclusion.

8. The first post-operative visit will occur one week after surgery. The next visit is planned to occur at the first dialysis visit which may occur between two and four weeks after surgery. This could result in a subject not being seen for three weeks after the initial assessment at the first postoperative week. A three-week interval is too long a duration without safety assessments being performed. Subjects should be seen and examined at two weeks post surgery irrespective of whether they undergo dialysis on that day. Please revise your protocol accordingly.

9. Subjects on dialysis will be monitored for flow assessment in their usual course of treatment. These assessments should be captured at frequent time points during your study until twelve months post surgery. Please incorporate more frequent assessments in your revised protocol.

10. The protocol text, monitoring chart, and consent form have discrepant information concerning when routine fistulography will occur. Please clarify when routine fistulography will occur and revise your submission so that the sections of the protocol are consistent.

11 In your discussion of safety endpoints, you propose to collect events related to loss of graft patency as efficacy endpoints rather than safety endpoints. However, you do not have a proposal for collecting these data in the efficacy endpoints. Please revise your protocol to collect data on loss of graft patency as adverse events and to include events related to loss of graft patency in your safety analysis.

12. You have proposed findings on physical examination (PE) and EKG as safety endpoints, but no PEs or EKGs are planned after screening. Please indicate at which study visits additional PEs and EKGs will be performed and incorporate this change into your protocol. Please ensure that the PE will capture findings relevant to potential adverse events.

13 You propose to have a Data Safety Monitoring Committee (DSMB) determine the safety of dose escalation. Please submit the DSMB charter that includes the guidelines by which the DSMB will assess the safety of your product for dose escalation.

14. Your product requires surgical application of a perivascular biodegradable collagen collar into which test material containing the adenoviral vector with the transgene product is injected. It is possible that unanticipated events involving placement of the collar or injection of test material into the collar may occur. Please include study-stopping rules in the protocol such that the occurrence of an excessive number of adverse events would necessitate stopping the study or expanding the dosing cohort for safety concerns.

15. Please submit a monitoring plan for your clinical trial as requested in the March 6, 2000 letter (available at http://www.fda.gov/cber/ltr/gt030600.htm). The plan should incorporate all the elements of the checklist found at http://www.fda.gov/ohrms/dockets/ac/01/briefing/3739b1_05_appendixE.htm.

You may not initiate clinical trials under this IND until your response to the above deficiency has been received and reviewed by FDA, and you are informed that the response is satisfactory. When you respond to all of the above issues, please identify your response as a "CLINICAL HOLD COMPLETE RESPONSE" and submit this information in triplicate to the IND. In addition, FAX a copy of the Form 1571, cover letter, and delivery tracking number to Patricia Hong at 301-827-0910. Your responses to any non-hold issues should be addressed in a separate amendment to the IND. For additional information, please refer to the FDA Guidance: Submitting and Reviewing Complete Responses to Clinical Holds - October 2000 (<http://www.fda.gov/cber/gdlns/clinhld1000.pdf>).

We also have the following comments and requests for additional information:

PRODUCT INFORMATION

16. To avoid the release of drug product that has not been properly tested or does not meet lot release specifications, we recommend that CoAs for the final drug product contain the following additional information:

    a. The specification for each lot release test, not just the result.

    b. Tests performed on the CVL and bulk purified drug substance, if these tests are required for the in-process material to be released for further processing to drug product. Examples of such tests include mycoplasma, *in vitro* adventitious virus, AAV, sterility on purified bulk drug substance, and RCA. Specifications and results should be included.

17 Regarding Trinam™ lot CTL2002#123U, according to the description of the manufacture and filling of this lot, bulk drug substance was simply thawed and aliquoted into vials to produce the final drug product. However, there are significant

differences in the viral particle concentration, infectious titer, pH of the bulk drug substance (see table 7-1A.20, section 7.1, p.85), and the final filled product (see Appendix 7-1.5, p. 161). Please clarify if the drug substance was further manipulated after thawing and before filling, and if not please explain the significant change in these test values. Also, we recommend that stability studies of in-process steps be put in place to study these variations.

18. The proposed "vascular protective" mechanism of VEGF-D supposedly includes inhibition of cellular proliferation in vascular smooth muscle. Please comment on the rationale for measuring stimulation of proliferation in rabbit aortic vascular smooth muscle cells transduced with Trinam™ Ad-VEGF-D as an assay of potency.

19 For final drug product that is manufactured by dilution of bulk drug substance with a diluent solution, we recommend that you repeat tests for pH and osmolality. Please comment.

20. We recommend that you set specifications for an acceptable range of pH. Please comment.

21. We recommend that you determine the required volume of sample to be removed for both in-process and lot release tests to avoid not performing tests (e.g., identity and bioburden) due to a lack of material. Please comment.

22. Please describe how the product will be shipped to the clinical site. Also, please provide data to support stability of the product under the shipping conditions proposed. Please be aware that prior to initiation of a Phase 3 study, shipping conditions will need to be fully validated. Please comment.

23 Please submit CoAs for reagents used during manufacture and purification of your Trinam™ product.

24. To support further product development, we recommend that a stability program be developed to establish storage conditions and a dating period for the final Trinam™ product. Stability protocols should include, but are not limited to, an analysis for product potency, evaluation of product integrity and sterility. Please refer to the FDA "Guideline for Industry: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (1996)" (ICHQ5C) found at http://www.fda.gov/cder/guidance/index.htm, as well as the FDA "Draft Guidance for Industry: Stability Testing of Drug Substances and Drug Products (1998)" for advice on the development of stability protocols which can be found at http://www.fda.gov/cber/gdlns/stabdf.pdf.

25. Please note that specifications should be set such that they can be achieved consistently. As an example, we recommend that a more realistic specification be set for the amount of CsCl present in your final product and that when this specification is not met you must not use this lot of product in clinical trials.

26. You have indicated that the source of the collagen used in the manufacture of the collagen collar and GAO kit portions of your treatment system is US cows. Please identify whether these cows come from a closed herd, what vaccinations are standard for the herd, how often does a veterinarian perform inspections of the herd, and whether the abattoir where the animals are slaughtered is USDA inspected and approved. Also, please address any possible viral contamination of the collagen source and provide assurance that any contaminating virus would be inactivated. Therefore, please provide information that supports the inactivation of four model viruses consisting of DNA, RNA (single and double stranded), and encapsulated and non-encapsulated viruses. We expect that a SAL of 10^{-6} can be supported. If this information is available in the cross-referenced master file, please identify the sections and pages.

27 Both the collagen collars and GAO kit have been described as meeting endotoxin specifications. Please provide the test protocol and results. If this information is available in the cross-referenced master files, please identify the section and page.

28 You have provided support for six month expiration dating for the collagen collar portion of the device, but you have not provided any expiration dating information for the GAO kit. Please provide information that supports any expiration date you intend to place on the collagen collar and GAO portions of your Trinam™ System. Please be advised that any accelerated aging studies must be supported with real-time data. If this information is available in the cross-referenced master files, please identify the section and page.

29. Please identify the regulatory status of the GAO kit.

30. You have described the GAO kit as being sterilized using ethylene oxide. Please describe how sterility is validated and evidence that sterility is achieved.

31 Please provide responses to questions 1-4 of the March 6, 2000 letter (available at http://www.fda.gov/cber/ltr/gt030600.htm) sent to all sponsor of gene therapy INDs. If your product is produced in a contract manufacturing facility you will need to obtain this information from the manufacturer. Alternatively, the manufacturer may submit this information to a master file. In addition, please submit yearly manufacturing updates that: 1) address all manufacturing information requested in items 1 through 4 of that letter; 2) affirm that manufacturing quality assurance (QA) and quality control (QC) and clinical trial oversight and monitoring have been conducted per the plans

submitted to FDA; and 3) submit modifications or updates to those plans as appropriate. For administrative convenience, we request that you provide this information in your annual reports.

CLINICAL INFORMATION

32. Regarding the consent form:

a. Please revise your consent form to accurately reflect the study procedures, including the possibility of archiving of blood samples and potential for future analysis.

b. Please revise the consent form to avoid misleading statements. Some examples of such statements include, but are not limited to the following:

1) The statement on page 1, "Once one amount has been shown to be safe, larger amounts will be given" implies that the safety of the lower amount is known. The word "once" should be replaced with the word "if."

2) Page 3 states that Trinam™ has been used successfully in pigs. This implies that efficacy can be assessed in a similar manner in pigs as in humans. The wording of the consent form should be revised to reflect that activity of the product was suggested by results of studies in pigs.

3) The statement on Page 3, "Persons with cancer should not take part in this study" should be changed to "Persons with a history of cancer, except non-melanoma skin cancer, should not take part in this study."

4) On page 4, there is a statement that fistulography will occur at 12 and 24 weeks. Please revise this statement so it is consistent with the protocol and monitoring chart.

5) The statement on page 4, "There are no approved treatment available so far that help access grafts last longer" is misleading. Grafts can be treated with thrombectomy or embolectomy if stenotic or closed, and this should be reflected in the consent form as an alternative treatment.

6) The discussion of risks on page 5 does not mention the death of a subject due to liver failure who had been given intrahepatic adenovirus, nor the occurrence of cancers (leukemias) in two children with severe combined immunodeficiency (SCID) who had been treated with hematopoietic stem cells transduced with a retroviral vector. Please add information concerning these adverse events to the consent form.

7) The discussion on page 5 of the risk of administration of a gene containing VEGF-D notes that VEGF-D has not been administered to people before and states "but it is not expected to cause problems." This phrase implies that there are no ill effects of VEGF-D. It is well documented that adverse effects, such as edema, have occurred with the administration of other members of this class of growth factor, and it may be misleading to suggest that no problems are expected with the administration of your product. We suggest that you remove this phrase from the consent form.

If you have any questions, please contact the Regulatory Project Manager, Patricia Hong, at (301) 827-5102.

Sincerely yours,

Stephanie Lisnek for JF

Joyce Frey, Ph.D.
Acting Deputy Office Director
Office of Cellular, Tissue,
and Gene Therapies
Center for Biologics
Evaluation and Research

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

Our Reference BB-IND 1010

JUL 16 2003

Ark Therapeutics Limited
Attention: James M. Parker, Ph.D.
Strategic Bioscience Corporation
93 Birch Hill Road
Stow, MA 01775-1308

Dear Dr. Parker:

We have reviewed the June 16, 2003 submission to your **Investigational New Drug Application (IND)** for "Adenovirus Vector (Ad-VEGF-D, Cobra-Bio Manufacturing) Expressing Human Vascular Endothelial Growth Factor D Gene; Delivered via Collagen Collar Device."

As discussed during the telephone conversation between you and Ms. Patricia Hong of this office on July 14, 2003, you have satisfactorily addressed the issues raised in our letter of May 30, 2003. The clinical hold has been removed and your proposed study may proceed. However, we have the following comments and requests for information:

PRODUCT INFORMATION

1 Regarding the RCA assay, please be aware that as an assay for final product purity and safety, this test is most appropriately performed after the vector has been purified. In addition, you have not supplied any data to support the assumption that the ratio of viral to infectious particles in the crude virus lysate is 30. As such, there is no evidence to support the claim that testing $1x10^9$ particle forming units (pfu) of the vector is equivalent to testing $3x10^{10}$ virus particles (vp). The current lots of Ad-VEGF-D, which have been tested for RCA in-process, may be used in your clinical trial. However, please modify your testing strategy so that all future lots of product are tested after the virus has been purified, the virus particle concentration of the final product has been determined, and the input number of virus particles into the assay can be empirically determined.

2. Please refer to the identity assay specifications listed in your original submission (Table 7-1A.19, p. 72). Currently these assays are performed on the drug substance (purified bulk). Please be aware that per 21 CFR 610.14 identity assays for licensed drug products are to be performed on samples of vialed drug product, after all filling and labeling operations have been completed. We recommend that you modify your identity assay to comply with 21 CFR 610.14 to ensure that the appropriate assay is in place by Phase 3 trial. Please comment.

3 Please be aware that per 21 CFR 610.1 each test required for lot release should be performed on samples of the lot after completion of all processes of manufacture which may affect compliance with the standard to which the test applies. We note that tests for potency, as well as certain tests for purity (such as residual host cell DNA and protein) are performed on the drug substance (purified bulk). We recommend that you collect data to demonstrate that manufacturing steps that are performed subsequent to sampling for these assays (such as dilution, filtering, and vialing) do not affect the assay outcome. Please be aware that by Phase 3 testing on the appropriate sample will need to be in place.

You are responsible for compliance with the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Please refer to the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (http://www.nih.gov/od/oba/) and the requirements for submissions to the NIH Office of Biotechnology Activities (OBA). Sponsors of human gene therapy protocols subject to the NIH Guidelines **are required to submit to NIH OBA all new clinical trial sites, all new protocols and protocol changes (including those initiated by the sponsor and those resulting from either FDA or the RAC review), and all serious adverse events.** Please refer to the November 5, 1999, Dear Gene Therapy IND Sponsor/Principal Investigator Letter (http://www.fda.gov/cber/ltr/gt110599.htm) and the Federal Register notice of amendments to the NIH Guidelines (65 FR 60328, http://www4.od.nih.gov/oba/1010frnotice.pdf). Reports submitted to NIH OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010. Contact NIH OBA at (301) 496-9838 for further information.

As requested in the FDA's March 6, 2000 letter to all gene therapy sponsors (available at http://www.fda.gov/cber/ltr/gt030600.htm), please submit yearly updates that: 1) address all manufacturing information requested in items 1 through 4 of that letter, 2) affirm that manufacturing QA and QC and clinical trial oversight and monitoring have been conducted per the plans submitted to FDA, and 3) describe modifications or updates to those plans as appropriate. For administrative convenience, we request that you provide this information in your annual reports.

If you have any questions, please contact the Regulatory Project Manager, Patricia Hong, at (301) 827-5102.

Sincerely yours,

Joyce L Frey

Joyce Frey, Ph.D.
Acting Deputy Office Director
Office of Cellular, Tissue,
and Gene Therapies
Center for Biologics
Evaluation and Research

Ark Therapeutics Limited
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A: INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN

1. INTRODUCTION

Hemodialysis access complications remain a major cause of morbidity for patients with end-stage renal disease who are undergoing chronic hemodialysis. Vascular access complications occur in approximately 40% of patients with polytetrafluorethylene (PTFE) grafts within the first 6 months, primarily due to stenosis and thrombosis. Thrombosis at the site of vascular access increases the risk of infection and the need for hospitalization, and may lead to loss of potential new sites for vascular access. To a large extent, the failure of hemodialysis access is due to the rapid development of an intimal hyperplastic lesion in the region of anastomosis between the PTFE graft and the vein. The hospital costs related to hemodialysis access procedures are estimated to be around $1.3 billion per year and the total cost of hemodialysis complications to the US healthcare system is thought to be in excess of $2 billion per year.

Ark Therapeutics Ltd is developing a product (Trinam™), for the prevention of *de novo* stenosis at the graft-vein anastomosis site in patients who require vascular access to facilitate hemodialysis for end-stage renal disease. Trinam™ is a vascular endothelial growth factor D (VEGF-D) gene in an adenoviral vector which is delivered locally to the adventitial surface of a graft-vein anastomosis by means of a biodegradable collagen collar device.

The rationale for Trinam™ to prevent *de novo* stenosis at the graft-vein anastomosis follows the discovery that VEGF has a 'vasculoprotective' action, resulting in inhibition of smooth muscle cell migration and proliferation[1]. The fundamental mechanism for this vasculoprotective effect of VEGF, as distinct from its more widely appreciated 'angiogenic' role, is that VEGF acts on surface receptors on endothelial cells resulting in increased production of nitric oxide and prostacyclin. These entities diffuse into the media of the blood vessel wall and counter stenosis development.

In an *in vivo* rabbit model of intimal thickening in carotid arteries, adventitial delivery of VEGF using a silastic collar as a gene delivery reservoir prevented smooth muscle cell proliferation. Further pre-clinical animal model work has shown that efficient gene transfection can be achieved in target tissues using adenoviral vectors. Finally, a study was undertaken to investigate the safety and efficacy of Trinam™ in an animal model using a pig, which mimics the human hemodialysis situation. The study demonstrated that Trinam™ was well tolerated up to and including a dose level of 1×10^{11} viral particles. In relation to efficacy, an effect on graft stenosis was observed, consistent with the magnitude of effect that is hoped for in humans.

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[1] Zachary I *et al.*
Vascular protection: A novel nonangiogenic cardiovascular role for vascular endothelial growth factor.
Arterioscler Thromb Vasc Biol 2000; 20(6): 1512-1520. Appendix 3.1.

The objective of the proposed study, which is the subject of this IND application, is to assess the safety and efficacy of ascending doses of Trinam™, when compared to controls, when applied locally to the graft-vein anastomosis site in patients with end-stage renal failure who require vascular access for hemodialysis. It is hypothesised that Trinam™ administration locally will result in less stenosis at the graft-vein anastomosis site (as measured by fistulography) compared to controls and, therefore, will reduce the need for interventions in dialysis patients. For this study, a single centre will enrol a total of 20 patients and these will be evaluated for efficacy over a six-month period and for safety over a twelve-month period. Patients will receive one of two dose levels by single administration locally of Trinam™, namely 4×10^9 viral particles or 4×10^{10} viral particles or will receive no treatment (i.e. standard surgery). The maximum dose that will be administered in this clinical study is less than the highest dose, 1×10^{11} viral particles, that was administered in the pre-clinical toxicology study in the pig model and at which no significant toxicology findings were observed.

2. BACKGROUND

Maintaining good vascular access for long-term hemodialysis remains a major clinical problem. Normal arteries in the arm or leg cannot be punctured repetitively for months or years, and normal veins would not accommodate the blood flow rates needed. For hemodialysis, it is necessary to gain access several times a week to large blood vessels capable of handling the substantial blood flow rates to and from artificial kidney dialyzers. Hemodialysis arteriovenous (AV) access is most commonly provided by constructing an internal AV fistula, an artery-vein anastomosis (typically of the radial artery and cephalic vein) or by placing a synthetic AV hemodialysis graft, which connects the artery to the vein. Most hemodialysis AV access grafts are expanded PTFE synthetic conduits, usually placed in loop configuration in the forearm (connecting the brachial artery to the anticubital cephalic or basilic vein) or in straight configuration in the forearm or the upper arm. The characteristics of the patient's arterial, venous and cardiopulmonary systems will influence which access type and location are most desirable for the patient. Malfunction of both types of permanent vascular access remains a leading cause of morbidity in patients who require chronic hemodialysis.

Only around 40% of PTFE vascular access grafts will remain patent and unobstructed for up to one year following installation[2]. The most common cause of vascular access dysfunction is thrombosis which is generally the result of venous stenosis[3, 4]. These stenoses are usually caused by the

[2] Cinat ME, Hopkins J, Wilson SE.
A prospective evaluation of PTFE graft patency and surveillance techniques in hemodialysis access.
Ann Vasc Surg 1999; 13(2): 191-198. Appendix 9.1.

[3] Kanterman RY *et al.*
Dialysis access grafts: anatomic location of venous stenosis and results of angioplasty.
Radiology 1995; 195(1): 135-139. Appendix 9.1.

progressive formation of neointimal hyperplasia in the venous outflow tract, most commonly at the graft-vein anastomosis[4].

Patients with access thrombosis and/or stenosis are at greater risk than patients with fully functioning grafts. Access thrombosis typically results in the use of temporary venous catheter placement, which is associated with infection and increased hospitalization rate. Venous stenosis reduces the efficiency of dialysis treatment, and predisposes to thrombosis. Furthermore, it is important to preserve vascular accesses due to the limited number of potential access sites[5]. Hemodialysis access related complications are the most common reason for hospitalization in patients with end-stage renal disease, accounting for 15-38% of their total hospital admissions[6, 7]. The hospital costs related to hemodialysis access procedures are estimated to be around $1.3 billion per year [United States Renal Data System annual report (USRDS)] and the total cost of hemodialysis complications to the US healthcare system is thought to be in excess of $2 billion. The National Kidney Foundation Dialysis Outcomes Quality initiative [NKF-DOQI] was established in recognition of the need to improve dialysis practice and outcomes. The NKF-DOQI has published clinical practice guidelines with recommendations for prospective graft surveillance in order to improve detection of vascular access dysfunction before thrombosis occurs[8]. Intervention with elective angioplasty or surgical revision once a hemodynamically significant stenosis has developed, reduces the rate of thrombosis and prolongs the average survival of the vascular access[4, 9].

Ark Therapeutics Ltd is developing a Vascular Endothelial Growth Factor (VEGF-D) gene therapy in an adenoviral vector which is delivered locally to the adventitial surface of a graft-vein anastomosis by means of a biodegradable collagen collar reservoir device. The proposed indication for

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[4] Palder SB *et al.*
Vascular access for hemodialysis. Patency rates and results of revision.
Ann Surg 1985; 202(2): 235-239. Appendix 9.1.

[5] Schwab SJ.
Assessing the adequacy of vascular access and its relationship to patient outcome.
Am J Kidney Dis 1994; 24(2): 316-320. Appendix 9.1.

[6] Chazan JA, London MR, Pono LM.
Long-term survival of vascular accesses in a large chronic hemodialysis population.
Nephron 1995; 69(3): 228-233. Appendix 9.1.

[7] Feldman HI, Kobrin S, Wasserstein A.
Hemodialysis vascular access morbidity.
J Am Soc Nephrol 1996; 7(4): 523-535. Appendix 9.1.

[8] K/DOQI Guidelines, 2001. Not supplied.

[9] Beathard GA.
Pecutaneous transvenous angioplasty in the treatment of vascular access stenosis.
Kidney Int 1992; 42(6): 1390-1397. Appendix 9.1.

this product (Trinam™) is the prevention of *de novo* stenosis at vascular surgical anastomoses. Trinam will initially be evaluated for safety and efficacy in prevention of stenosis at the graft-vein anastomosis site in patients with end stage renal disease who require vascular access for hemodialysis. The requirement for a surgical procedure to place a new PTFE vascular access graft will allow the perivascular collagen collar to be sealed in place around the graft-vein anastomosis for site-specific delivery of the VEGF gene.

Early phase clinical trials have demonstrated that vascular gene therapy is safe and well tolerated[10]. For example, adenoviral gene transfer to human peripheral arteries *in vivo* was found to be feasible and safe in a preliminary clinical study[11]. The study was performed in patients with lower leg ischemia scheduled to undergo amputation and increasing concentrations of adenoviruses (1×10^8 to 4×10^{10} viral particles) containing LacZ marker gene were administered into the arteries by catheter. Gene transfer efficiency ranged from 0.04% to 5% and was related to adenoviral titer. Successful gene transfer has also been observed in a similar study by the Sponsor, in which patients about to undergo amputation were treated with a liposome vector, containing VEGF-A encoding plasmids delivered with the biodegradable collagen collar used in Trinam™. Local VEGF gene transfer has also been studied in patients with angina[12]. Catheter-mediated, local VEGF plasmid/liposome gene transfer to human coronary arteries was performed immediately after angioplasty. This study demonstrated that gene transfer to coronary arteries was feasible and well tolerated with minimal systemic distribution of the VEGF transgene.

No pharmacological agent has yet been proven to prevent IHP or stenosis formation. It is hypothesized that Trinam™ will reduce the degree of stenosis in patients with end-stage renal disease, which would result in a fewer interventions to restore graft patency, a reduction in the incidence of thrombosis and a reduced need for replacement grafts. Trinam™ may therefore provide a significant therapeutic advance for a serious condition where no approved prophylactic treatment exists.

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[10] Yla-Herttuala S, Martin JF.
Cariovascular gene therapy.
Lancet 2000; 355: 213-222. Appendix 3.1.

[11] Laitinen M *et al.*
Adenovirus-mediated gene transfer to lower limb artery of patients with chronic critical leg ischemia.
Hum Gene Ther 1998; 9: 1481-1486. Appendix 3.1.

[12] Laitinen M *et al.*
Catheter-mediated vascular endothelial growth factor gene transfer to human coronary arteries after angioplasty.
Hum Gene Ther 2000; 11: 263-270. Appendix 9.1.

3. RATIONALE FOR THE CHOICE OF TRANSGENE AND VECTOR

VEGFs are a family of angiogenic growth factors that regulate multiple biological functions in the endothelium. Alternative splicing of human VEGF mRNA from a single gene containing eight exons gives rise to several different isoforms. The idea that VEGF can prevent the formation of neointimal proliferation at the graft-vein anastomosis stems from the discovery by scientists at Ark Therapeutics that VEGF has a 'vasculoprotective' action, such that VEGF inhibits smooth muscle cell migration and proliferation and thereby prevents *de novo* stenosis from occurring[1]. The fundamental mechanism for this vasculoprotective effect of VEGF, is distinct from its more widely appreciated 'angiogenic' role. Several investigators have established that VEGF stimulates endothelial production of nitric oxide (NO) and prostacyclin (PGI_2), intercellular mediators that are predicted to have vascular protective effect, including anti-proliferative effects in smooth muscle cells (SMCs), anti-platelet actions and in the case of NO, inhibition of leukocyte interactions with endothelium. The antimitogenic effects of NO and PGI_2 on SMCs have been demonstrated *in vitro* and *in vivo* acting via the intracellular messengers cGMP and cAMP respectively.

Three receptors for VEGF have been identified, VEGFR-1, VEGFR-2 and VEGFR-3. They are structurally related to the platelet derived growth factor (PDGF) family of receptor tyrosine kinases and have a similar domain structure. The complex interaction of the VEGFs and their receptors is not yet fully defined, but what has become evident is that the different binding affinities of the various isoforms to the three receptors elicit different profiles of effect. While all the VEGFs are capable of eliciting vascular protective effects, these may be accompanied by varying degrees of angiogenic and lymphangiogenic effects. Thus, it is now becoming possible to develop VEGF based gene medicines using the VEGF isoform with the profile of effect that is most appropriate to the indication being treated.

Ark Therapeutics' initial work used VEGF-A and then switched to the more recently discovered VEGF-D. VEGF-D is synthesized and secreted as a large precursor form, which is proteolytically processed into the mature form comprising the central VEGF homology domain. VEGF-D does not bind to VEGFR-1, the unprocessed form preferentially signals through VEGFR-3 and only the mature form efficiently triggers VEGFR-2 signaling. This produces an effect profile of VEGF-D in its full-length form, as used in Trinam™, that

it is weakly angiogenic[13], pro-lymphangiogenic[14] and vasculoprotective[1]. For the prevention of *de novo* stenosis at graft:vein anastomoses this profile is more appropriate than that of the strongly angiogenic VEGF-A isoform. This has been confirmed in a recent study conducted using adenoviruses injected into the hind limbs of rabbits where it was shown that Ad-VEGF-D had some stimulatory effect on capillary enlargement (2.2-fold) but did not enhance plasma protein extravasation significantly. In contrast, Ad-VEGF-A caused an increase in capillary density and a corresponding capillary enlargement of 6.4-fold coupled with a 13-fold increase in plasma protein extravasation[14].

For exogenously delivered VEGF gene sequences to be able to mediate a significant vasculoprotective effect, they must be delivered efficiently to the target cells. Initial work in rabbits[15] and humans [Study 101, described later in this submission] using plasmids encoding VEGF formulated in liposomes showed that the efficiency of gene delivery and hence the level of protein expression was low. Subsequently, a direct comparison of liposomal, retroviral and adenoviral delivery of VEGF sequences to rabbit carotid arteries *in vivo*, showed that adenoviruses were the most efficient mode of gene delivery[16]. Therefore, an adenoviral vector encoding VEGF-D was constructed for use in Trinam™.

4. SUMMARY OF THE DEVELOPMENT OF TRINAM™

Over the past four years, a large amount of research and development work has been undertaken to support the use of Trinam™ in the proposed indication and a structured development path has been followed. An outline of the preclinical research and development work and the completed and planned clinical development is presented in Figures 3A.1 and 3A.2 respectively.

Initially, a "proof of concept" study was performed in a rabbit model of IHP[15]. This study showed that a VEGF gene (VEGF-A in this instance) in a liposomal vector inhibited IHP of the carotid artery in rabbits through peri-adventitial administration via a silastic collar. The beneficial effect of VEGF

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[13] Joukov *et al.*
A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinase. Embo J 1996; 15: 290-298. Not supplied.

[14] Rissanen *et al.*
VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscles via adenoviruses. Circ Res (2003) In press. Not supplied.

[15] Laitinen M *et al.*
VEGF gene transfer reduces intimal thickening via increased production of nitric oxide in carotid arteries. Hum Gene Ther 1997a; 8(15): 1737-1744. Appendix 8.1.

[16] Laitinen M *et al.*
Gene transfer into the carotid artery using an adventitial collar: comparison of the effectiveness of the plasmid-liposome complexes, retroviruses, pseudotyped retroviruses, and adenoviruses. Hum Gene Ther 1997b; 8(14): 1645-1650. Appendix 8.2.

Figure 3A.1: Diagrammatic Representation of the Pre-Clinical Research and Development Program to support the use of Trinam™

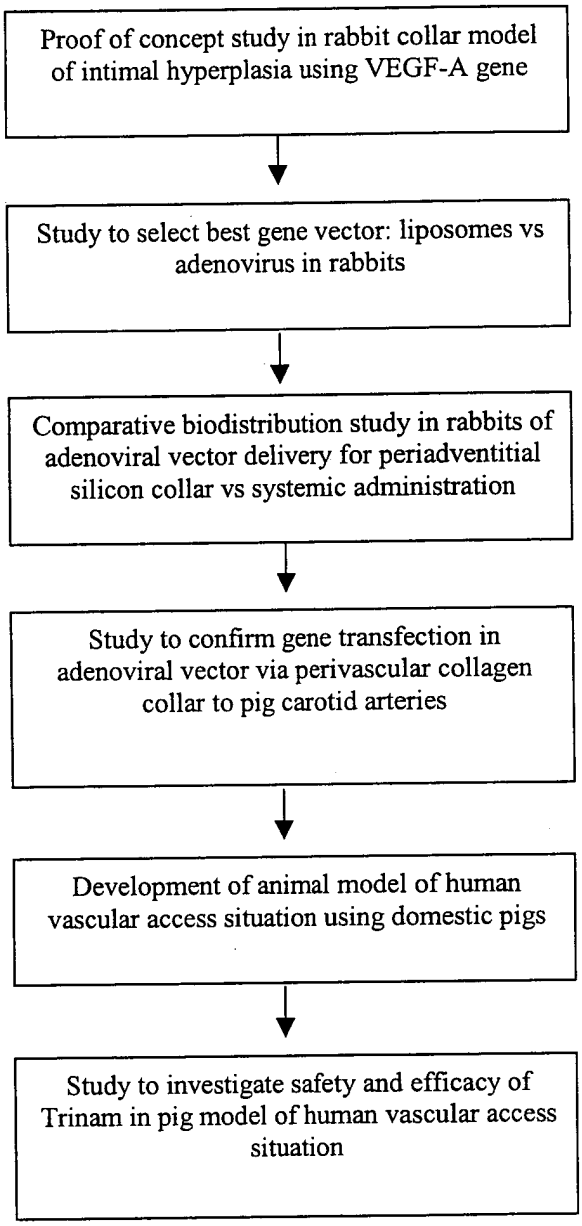
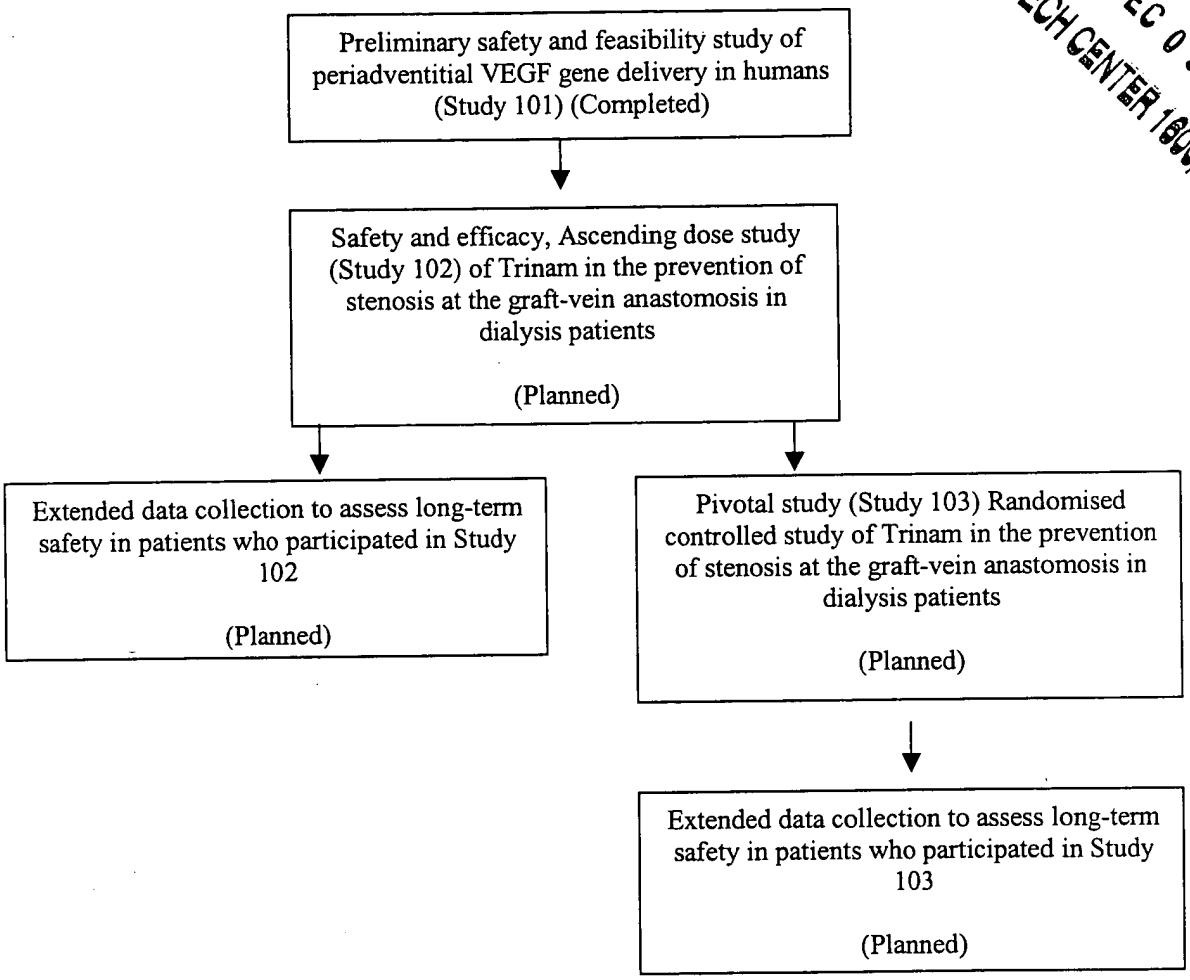


Figure 3A.2: Diagrammatic Representation of the Clinical Development Program to support the use of Trinam™



gene transfer was abolished by an inhibitor of NO synthase (N-nitro-L-arginine methylester [L-NAME]) suggesting that the effect was mediated by NO production.

Further work, using the rabbit collar model, was conducted to establish whether liposomes or an adenoviral vector would be the most appropriate gene delivery vehicle to take forward into full development[16]. This study showed that LacZ marker gene delivery with an adenoviral vector was more efficient than with a liposome when administered peri-adventitially via a silastic collar. As a result of its efficient delivery, the adenoviral vector was chosen as the vector for use in Trinam™.

A study was then undertaken to investigate the potential biodistribution of the adenoviral vector when administered via the perivascular collar device. This study, performed in rabbits, compared the biodistribution of an adenoviral vector when given via the silicon collar with that obtained by systemic administration[17]. The results demonstrated that gene transfer was focused at the site of delivery and that the biodistribution of the adenoviral vector to non-target tissues was considerably reduced by the use of the collar for peri-adventitial delivery when compared to delivery via the systemic route.

Although it had been shown that local gene delivery could be effected using a collar device, it was known that the silicon collars used in the rabbits and which are not biodegradable, would be unsuitable for use in Trinam™. The material chosen for collar construction in Trinam™ is collagen and an additional study was undertaken to confirm gene transfection using prototype collagen collars in pigs. In this study, collagen collars were placed around the carotid artery of a small number of pigs and a marker gene in an adenoviral vector was injected into the collar reservoir space, as shown diagrammatically below[18]. This study showed that the smooth muscle cells of the carotid artery were efficiently transfected with the adenoviral vector using the Trinam™ peri-adventitial delivery system (i.e. the biodegradable collagen collar intended for clinical use).

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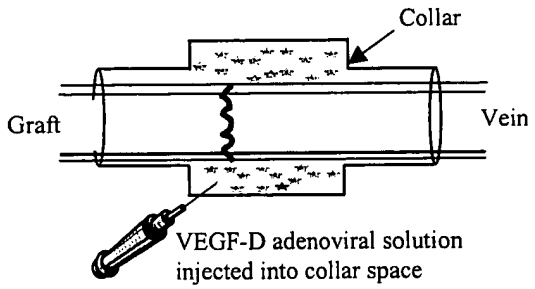
[17] Hiltunen M *et al.*
Biodistribution of adenoviral vector nontarget tissues after local *in vivo* transfer to arterial wall using intravascular and peri-adventitial gene delivery.
FASEB J 2000; 14: 2230-2236. Appendix.8.3.

[18] Pakkanen TM *et al.*
Peri-adventitial lacZ gene transfer to pig carotid arteries using a biodegradable collagen collar or a wrap of collagen sheet with adenoviruses and plasmid-liposome complexes.
J Gene Med 2000; 2(1): 52-60. Appendix 8.4.

Figure 3A.3



Having established targeted and biologically effective gene delivery, further pre-clinical work was undertaken to establish an animal model of the human vascular access situation for hemodialysis. In this model using domestic pigs, a PTFE hemodialysis graft was inserted surgically between the internal carotid artery and internal jugular vein. The pigs used in the model developed IHP at the site of the graft-vein anastomosis, similar to that seen in humans. The presence of IHP was confirmed by ultrasound diagnosis and subsequent histological examination. It should be noted however, that the IHP that does develop in this model does so very rapidly and is evident between 7 to 14 days. Similar IHP lesions were seen in other studies conducted in pigs where PTFE grafts were placed end-to-side between either the iliac artery and vein[19] or the femoral artery and vein[20]. Also, stenosis forms at the venous anastomosis of sheep arteriovenous grafts within 4 to 8 weeks[21].

Using the pig model of the human vascular access situation, a comprehensive study was peformed to investigate the safety and efficacy of Trinam™. The study demonstrated that Trinam™ treatment up to and including a dose level of 1×10^{11} viral particles did not result in any systemic toxicity as assessed in terms of clinical health, weight gain, food consumption, clinical pathology, *post-mortem* observations and histological examination.

Also, quantitative PCR results at 3, 14 and 56 days showed no tissue biodistribution of the vector at doses of $1x10^{7}$ and $1x10^{9}$ viral particles and only minimal biodistribution at the highest dose of $1x10^{11}$ viral particles. At the site of administration (therapeutic site) 3 days after treatment, the PCR

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19 Johnson MS *et al.*
The porcine hemodialysis access model.
J Vasc Interv Radiol 2001; 12: 969-977. Appendix 8.12.

20 Kelly BS *et al.*
Aggressive venous neointimal hyperplasia in a pig model of arteriovenous graft stenosis.
Kidney International 2002; 62: 2272-2280. Appendix 8.12.

21 Kohler TR *et al.*
Dialysis access failure: A sheep model of rapid stenosis.
J Vasc Surg 1999; 30: 744-751. Appendix 3.1.

signal showed a correlation with virus dose. The magnitude of the signal declined with time, but the relationship with dose was maintained. Thus, analysis of tissues using quantitative PCR showed that the collar was effective in confining the therapeutic agent to the target tissue site (venous anastomosis) to achieve local delivery. As expected, where a signal was detected it declined with time, demonstrating viral clearance.

In relation to efficacy, the study findings showed that the administration of the lower dose of 1×10^9 viral particles did not produce any result that was appreciably different from the controls. However, at a dose of 1×10^{11} viral particles, a reduction in graft stenosis was observed, as measured by colour Doppler ultrasound. This effect on graft stenosis is consistent with the magnitude of effect that is hoped for in humans.

Study reports and/or publications of all the preclinical work are included in this submission.

Finally, prior to commencing the proposed clinical study in the target population, a preliminary safety and feasibility study (Study 101) was undertaken to evaluate peri-adventitial delivery of VEGF in man. This 'first in man' study was performed in a small number of patients with severe peripheral vascular disease, undergoing above-knee amputation, in order to assess gene transfection efficiency and safety following delivery of $VEGF_{165}$ (VEGF-A) using the perivascular collagen collar. This study was performed using VEGF-A encoding plasmids formulated in a liposome. Although gene transfer to the artery was achieved in all subjects, the development of VEGF-A encoding plasmids in liposomes for use in this indication was discontinued, for the reasons described above, in favour of VEGF-D encoding recombinant adenoviruses.

B: BROAD OBJECTIVES OF THE PROPOSED CLINICAL STUDY

The proposed clinical study (Study 102) will be performed in the target patient population and will evaluate the safety and efficacy of Trinam™ in stenosis prevention in patients who require vascular access for hemodialysis. Based on the the preclinical information obtained, it is hypothesized that the use of Trinam™ will lead to a clinically significant reduction (i.e. 25%) in the level of stenosis at the site of the anastomosis within 6 months and that the treatment will be well tolerated.

This is a phase II, open label, controlled, upward titration of 2 dosage levels. The study comprises a one-time treatment administered at the time of surgery for the individual patients. Eight patients will receive the first dose level of the treatment, followed by an evaluation period of one month. At the end of this period a decision to proceed to the next dose level will be taken. A Data Safety Monitoring Board (DSMB) composed of individuals who are independent of scientific or financial interests in the study will review all the safety data during the course of the study. Dose escalation will only occur with the agreement of the DSMB. Patients will be monitored for 12 months and after their involvement in the study has ended, all

patients will be required to agree to an extended period of monitoring to comply with current Gene Therapy Patient Tracking System guidelines (CBER 27 June 2002).

Enrollment will be in three discrete groups; Group 1 (8 individuals) will be treated with Trinam™ $4x10^9$ VEGF-D adenovirus particles, Group 2 (8 individuals) will receive Trinam™ $4x10^{10}$ VEGF-D adenovirus particles and Group 3 (4 individuals) will serve as the control group and have graft placement surgery without Trinam™ application. Recruitment into the control group will run in parallel with that into the active treatment groups.

The VEGF-D adenovirus solution will be injected into the reservoir of the biodegradable perivascular collagen collar after the collar has been applied to the anastomosis site (end to end) and sealed with collagen surgical sealant. The maximum dose that will be administered in this clinical study is less than the highest dose (1×10^{11} viral particles) that was administered in the pre-clinical toxicology study in the pig model and at which no significant toxicology findings were observed.

The safety and tolerability of Trinam™ treatment will be assessed through the following endpoints:

- Incidence of adverse events (AEs) (interventions due to loss of graft patency will not be defined as AEs as these will be captured as efficacy endpoints).
- Physical examination findings.
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature).
- 12-Lead electrocardiogram (ECG) measurements.
- Clinical laboratory test results (blood chemistry and hematology [urinalysis will not be measured since the patients will usually be anuric]).
- Levels of antibodies against adenovirus in plasma.
- Biodistribution of the adenoviral vector containing the VEGF-D gene in WBC and plasma by polymerase chain reaction (PCR).
- Ophthalmic evaluation performed by fundoscopy and documented by photography.

Although this is primarily a safety study, efficacy data will also be collected. The endpoints for this will be:

- Mean change in access flow rate from baseline (first dialysis visit using the new graft-vein anastomosis) to 6 months after surgery, as measured by a dilution technique (eg, ultrasound, conductance, or thermal dilution) or by Doppler flow.
- Percent stenosis at 6 months after surgery at the area of greatest narrowing of the graft-vein anastomosis, as measured by fistulography.